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Biochimica et Biophysica Acta 1407 (1998) 51–60

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

The form of iron oxide deposits in thalassemic tissues varies between different groups of patients: a comparison between Thai β-thalassemia/hemoglobin E patients and Australian β-thalassemia patients

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Received 6 January 1998; revised 8 April 1998; accepted 17 April 1998

Abstract

Mössbauer spectra of 12 β-thalassemia/hemoglobin E spleen samples from Thai patients who had not received multiple blood transfusions and chelation therapy and seven β-thalassemia spleen samples from Australian patients who had received multiple blood transfusions and chelation therapy were recorded with sample temperatures of 78 K. Each spectrum was found to consist of a superposition of a relatively intense central doublet characteristic of high-spin Fe(III), a low intensity sextet of peaks due to magnetic hyperfine-field splitting, and occasionally a doublet that could be attributed to heme iron. A significant ($P = 0.01$) difference (Kolmogorov-Smirnov statistic of 0.71) between the distributions of sextet signal intensity as a fraction (F_s) of the total non-heme iron Mössbauer spectral signal for the two groups of patients was detected. The distribution of F_s for the Thai β-thalassemia/hemoglobin E spleens had a mean value of 0.128 (S.D. 0.035) while that for the Australian β-thalassemia spleens had a mean of 0.27 (S.D. 0.12). No significant difference between the distributions of non-heme iron concentrations in the tissues for the two groups of patients was detected by atomic absorption spectrometry. This study shows that the Australian β-thalassemia patients had a higher fraction of their non-heme spleen iron in a goethite-like form than the Thai β-thalassemia/Hb E patients.

Keywords: Hemosiderin; Iron overload; Mössbauer spectroscopy; Thalassemia; Spleen; Iron

1. Introduction

Diseases such as the thalassemias and genetic hemochromatosis are examples of iron overload diseases. In the case of hemochromatosis the iron overload is a primary symptom of the disease in that there is a genetic defect [1,2] resulting in a loss of control of iron absorption from the diet. Once diagnosed, the disease can be effectively treated by regular phlebotomy to remove iron. In the case of tha-

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0925-4439/98/$19.00
PII: S0925-4439(98)00026-X

lassemia, there is a genetic defect resulting in ineffective synthesis of hemoglobin leading to anemia [3]. The anemia, if left untreated, leads to increased erythropoiesis and increased absorption of iron from the diet [4]. The increased iron absorption results in iron overload. Most cases of thalassemia major in wealthy countries are treated with regular red cell transfusions (to alleviate the anemia). The blood transfusions result in normal rates of erythropoiesis and iron absorption from the diet but in themselves constitute a large influx of iron to the body. Thus, iron chelation therapy is normally administered in conjunction with red cell transfusion therapy. In less wealthy countries, however, thalassemic patients may receive few, if any, blood transfusions and no chelation therapy.

In both thalassemia and hemochromatosis, excess iron is deposited in the tissues in the form of ultrafine particles of iron(III) oxyhydroxide [5]. At low levels of loading, the iron(III) oxyhydroxide particles are mostly found within the iron storage protein, ferritin. Ferritin is an approximately spherical molecule with a central cavity within which the iron(III) oxyhydroxide particle is held [6]. The diameter of the cavity is about 8 nm. The protein shell, which is approx. 2 nm thick, renders the particle water soluble. At higher levels of iron loading, iron(III) oxyhydroxide particles are mostly found in insoluble aggregates associated with protein residues. This form of deposit is known as hemosiderin [7]. High levels of iron deposits are toxic to the cell, causing cell damage through reactions such as catalysis of lipid peroxidation.

The structures of the mineral particles within mammalian ferritins have been found to be based on that of the mineral ferrihydrite ($5Fe_2O_3{\cdot}9H_2O$) [8] with variations in the degree of crystallinity and particle size being observed for ferritins from different human organs and disease states [9]. However, three quite different iron(III) oxyhydroxide mineral structures have been identified in hemosiderins from different human tissues in several case studies [5,10–12]. The three different structures are similar to (i) the mineral ferrihydrite, (ii) a highly defect and poorly crystalline goethite (α-FeOOH), and (iii) non-crystalline hydrated iron(III) oxyhydroxides. Preliminary studies have suggested that there may be relationships between the mineral form of the iron(III) oxyhydroxide particles of hemosiderin and the type of disease and/or the clinical treatment administered to the patient [5,10,11,13–15]. However, insufficient numbers of patients in these case studies prevented confident conclusions being drawn about such relationships. In fact, a more recent study has questioned the validity of the proposed relationships [12] based on two further case studies. Thus the question of whether the mineral structure of hemosiderin iron deposits is determined by the type of disease, the clinical treatment administered, or some other factor has still not been satisfactorily answered. The present study aims to elucidate these questions by studying the form of iron(III) oxyhydroxide deposits in a relatively large number of patients from two quite distinct and identifiable groups, namely (i) seven Australian β-thalassemic patients who have received multiple transfusions of packed red cells and regular chelation therapy and (ii) 12 Thai β-thalassemia/hemoglobin E patients who have received few, if any, red cell transfusions and no chelation therapy.

2. Materials and methods

Samples of spleen were obtained from seven Australian β-thalassemic patients who had undergone splenectomy at Princess Margaret Hospital, Perth. The β-thalassemia trait was determined by typing of the hemoglobin using electrophoresis in combination with standard hematological techniques. The ages of the patients at the time of splenectomy ranged from 10 to 15 years and all had received regular transfusions of packed red cells together with chelation therapy. Regular transfusions of packed red cells (15–20 ml/kg body mass/month) were started at about 12 months of age. This is equivalent to a rate of approx. 0.5 units of packed red cells per month at age 1 year increasing to 1–2 units of packed cells per month by the age of approx. 10 years. Chelation therapy using desferrioxamine (Desferral, Ciba-Geigy) was started between the ages of 2 and 3 years. Samples of spleen, liver and pancreas were also obtained either post splenectomy or post mortem from 12 Thai β-thalassemia/hemoglobin E patients from Siriraj Hospital, Bangkok. The β-thalassemia/hemoglobin E trait was also deter-

mined by typing of the hemoglobin using electrophoresis in combination with standard hematological techniques. The age of the patients ranged from 20 to 40 years and they had received few (less than a total of 30 units of packed red cells), if any, red cell transfusions and no chelation therapy.

Tissue samples were freeze dried and ground to a powder prior to iron content analysis with atomic absorption spectrometry and Mössbauer spectroscopy.

Mössbauer spectra were recorded in transmission geometry using a ^{57}Co in rhodium foil source. The source was driven at constant acceleration from −13 to +13 mm/s with a double ramp waveform. Spectra were subsequently folded to eliminate the parabolic background due to variation in the solid angle subtended by the detector window about the source. The resulting spectra consisted of 250 data points with a background count of between 0.3 and 1.5×10^6 per channel. The counting time was determined by the properties of the sample under study (see later). The velocity scale was calibrated with reference to the spectrum of an α-iron foil at room temperature, the center of the spectrum being taken as zero velocity. Freeze dried samples of tissue were packed into 10 mm diameter perspex sample holders. The thickness of the sample was adjusted so that the 14.4 keV Mössbauer γ-rays were attenuated by approx. 1/*e*. Sample temperatures were maintained at 78 K during the spectral data acquisition using a liquid nitrogen cryostat.

Each Mössbauer spectrum was initially fitted with a doublet and sextet of Lorentzian peaks using a sum of squares minimization procedure. In some spectra, a signal due to heme iron was detectable and as such a second doublet was included in the fitting procedure. The two peaks in each doublet were constrained to be of equal area and width while the ratio of areas of the outer to middle to inner pairs of peaks of the sextet were constrained to be in the ratio 3:2:1. The linewidths, center shift, and quadrupole splitting of the doublet were allowed to vary freely during the fit as were the linewidths, magnetic hyperfine-field splitting, center shift, and quadrupole perturbation on the sextet. This fitting procedure yields reproducible spectral parameters and doublet to sextet area ratios for spectra with high signal to noise ratios. However, for spectra with low sextet-signal to noise ratios, this fitting procedure tends to give unreliable results.

Mean values for the Mössbauer spectral parameters of the sextet signal in the four spleen spectra with the highest sextet-signal to noise ratio were used as a standard with which to refit all of the spleen tissue spectra. All of the spleen spectra were refitted with a high-spin Fe(III) doublet, a heme doublet where necessary, and the standard sextet. During the fitting procedure, the linewidths, magnetic hyperfine-field splitting, center shift, and quadrupole perturbation of the sextet were allowed to vary by up to 1 S.D. from the mean values derived from the four spectra with the highest sextet-signal to noise ratios. The area of the sextet was allowed to vary freely. In this way the relative spectral area of the sextet in spectra with very low sextet-signal to noise ratios could be more reliably assessed assuming that the Mössbauer spectral parameters of the sextet do not vary significantly from sample to sample.

In order to gauge the magnitude of the error in measurement of the relative spectral area of the sextet component in the spectra, the spectra were analyzed in terms of the total numbers of counts within particular velocity regions of interest. Three regions of interest were identified by inspection of the four spleen spectra with the highest sextet-signal to noise ratio. The three regions represent (i) velocity regions where the Mössbauer signal is negligible (i.e. background), (ii) velocity regions encompassing almost all of the outer peaks of the sextet without encompassing any significant intensity from other parts of the spectrum, and (iii) the entire velocity range of the spectrum. These regions of interest can be defined by four velocity boundaries which were found by inspection to be $v_1=-9.0$ mm/s, $v_2=-5.5$ mm/s, $v_3=6.2$ mm/s, and $v_4=9.5$ mm/s where region (i) is $v<v_1$ or $v>v_4$, region (ii) is $v_1<v<v_2$ or $v_3<v<v_4$, and region (iii) is the entire velocity scan of the spectrum ($v_{\min}<v<v_{\max}$). If c_i is the number of data channels in region i, X_i is the total number of counts detected in region i, B_i is the total number of counts expected in region i if the intensity of the Mössbauer signal were zero, and $S_i=B_i-X_i$ (i.e. the number of counts 'missing' in region i due to the presence of the Mössbauer signal) then the fractional area of the spectrum in the sextet is given by

$$s = \frac{2S_2}{S_3} \pm \frac{2S_2}{S_3}\left[\frac{\left(\frac{c_2^2 X_1}{c_1^2} + X_2\right)}{S_2^2} + \frac{\left(\frac{c_3^2 X_1}{c_1^2} + X_3\right)}{S_3^2}\right]^{1/2} \quad (1)$$

assuming that the ratio of areas of the outer to middle to inner pairs of lines in a sextet is 3:2:1 and that any signal in region 2 is solely due to a sextet similar to the ones observed in the spectra with high sextet-signal to noise ratios. The error expression in Eq. 1 is obtained by commutation of Poisson statistical errors of counting in each region of interest.

Thus this method of analysis gives an indication of both the relative spectral intensity of a sextet in the Mössbauer spectra of spleen tissues and the inherent error on the measurement due to the Poisson statistics of counting. This is particularly useful when the signal to noise ratio is very low.

3. Results

Iron concentrations in the spleen samples were measured using atomic absorption spectroscopy and are shown in Table 1. A Kolmogorov-Smirnov (K-S) test on the data did not indicate any significant difference between the distribution of iron concentrations in the Thai β-thalassemia/Hb E spleens and the Australian β-thalassemia spleens.

Mössbauer spectra of the spleen samples were recorded with samples at 78 K. All spectra showed a relatively intense central doublet (Fig. 1) with spectral parameters (Table 2) indicative of paramagnetic or superparamagnetic high-spin Fe(III). Many of the spectra also clearly showed a sextet signal (Fig. 1b). Some spectra showed an additional low intensity doublet which could be attributed to heme iron. By assuming equal recoil-free fractions for the different iron atoms in the tissue at 78 K, the fraction of heme iron in the samples was calculated thus enabling the non-heme iron concentrations in the tissues to be deduced (Table 1). Since the heme signal in each spectrum was very small, if detected at all, the values for the non-heme iron concentrations were not very different from those for the total iron concentrations. A K-S test on the data did not indicate any significant difference between the distributions of non-heme iron concentrations in the Thai β-thalassemia/Hb E spleens and Australian β-thalassemia spleens.

Table 2 shows the mean values and standard deviations for the Mössbauer spectral parameters of the non-heme iron doublet signal for the Thai β-thalassemia/Hb E and Australian β-thalassemia spleens. Previously reported data for normal spleens are also shown [16]. K-S tests on the distributions of spectral parameters in each group showed significant differences between (i) the quadrupole splittings for the Thai β-thalassemia/Hb E and the normal spleens (K-S statistic = 1.00, $P < 0.01$), (ii) the quadrupole splittings for the Australian β-thalassemia spleens and the normal spleens (K-S statistic = 1.00, $P < 0.01$), (iii) the center shifts for the pooled thalassemia spleens (Thai plus Australian) and the normal spleens (K-S statistic = 0.52, $P = 0.02$). Differences were also identified, but with a lower degree of confidence, between the distributions of (iv) the center shifts for the Thai β-thalassemia/Hb E spleens and normal spleens (K-S statistic = 0.50, $P = 0.07$), (v) the

Table 1
Mean values and standard deviations (or ranges) for iron concentrations (mg Fe/g dry tissue), non-heme iron concentrations (mg Fe/g dry tissue), and fractions, F_s, of the non-heme Mössbauer spectral signal in the form of a sextet for human tissue samples

Tissue	Iron concentration	Non-heme iron concentration	F_s
Thai β-thal/Hb E spleen ($n = 12$)	5.5	5.4	0.128
S.D.	1.7	1.8	0.035
Aus β-thal spleen ($n = 7$)	7.8	7.7	0.27
S.D.	6.0	6.0	0.12
Thai β-thal/Hb E liver ($n = 4$)	32	32	0.11
Range	18–43	18–43	0.08–0.14
Thai β-thal/Hb E pancreas ($n = 3$)	16	16	0.17
Range	12–20	12–20	0.12–0.27

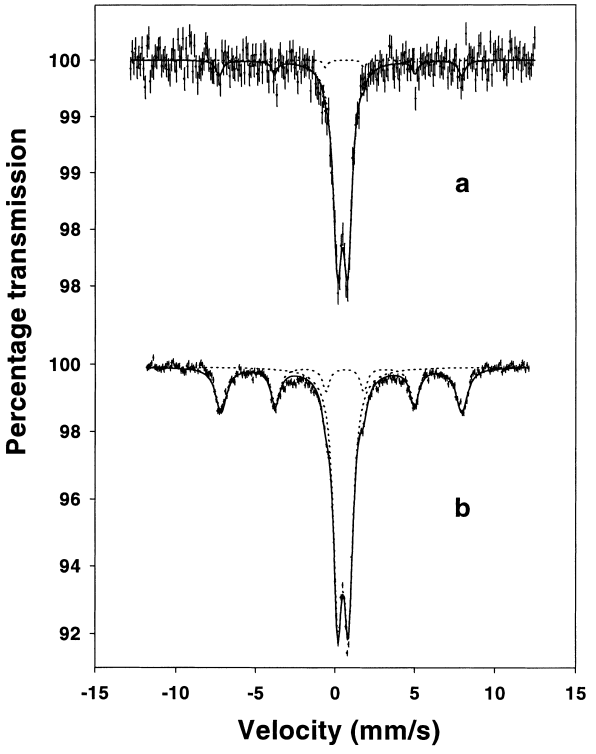

Fig. 1. Mössbauer spectra of (a) a sample of Thai β-thalassemia/Hb E spleen and (b) a sample of Australian β-thalassemia spleen at 78 K. The size of the error bars on each data point is given by $\sqrt{N}$ where N is the number of γ-ray counts for that data point. The solid curves are least squares fits of the central doublet and standard sextet components to the spectrum (see Section 2). The dotted curves indicate the two spectral subcomponents comprising the fit. (Heme signals were not detectable in either of these two spectra.)

center shifts for the Australian β-thalassemia spleens and the normal spleens (K-S statistic = 0.55, $P = 0.09$), and (vi) the linewidths for the Australian β-thalassemia spleens and normal spleens (K-S statistic = 0.58, $P = 0.06$).

In many of the spleen tissue Mössbauer spectra the sextet-signal to noise ratio was low and the free fitting of Lorentzian peaks to the data often gave unreliable results due to peaks being fitted to the noise rather than true signals. In order to measure more reliably the fraction, *s*, of the Mössbauer spectrum made up by the sextet signal, it was assumed that the parameters of the sextet signal were very similar for all spleen samples so that a standard sextet with constrained parameters could be fitted to the data. The parameters of the standard sextet were taken to be the mean values of the spectral parameters of the four spectra with the highest sextet-signal to noise ratio (Table 3). All of the spleen spectra were then refitted with a high-spin Fe(III) doublet, a heme doublet where necessary, and the standard sextet. During the fitting procedure, the linewidths, magnetic hyperfine-field splitting, center shift, and quadrupole perturbation were allowed to vary by up to one standard deviation from the mean values derived from the four spectra with the highest sextet-signal to noise ratios. The sextet signal intensities as a fraction (F_s) of the total non-heme iron Mössbauer spectral signal derived from this method of fitting are given in Table 1. The heme signal was typically less than 5% of the

Table 2
Mean values and standard deviations (or ranges) for Mössbauer spectral parameters of the central doublet components of human tissues at 78 K

Tissue	δ	ΔE_Q	Γ
Thai β-thal/Hb E spleens ($n = 12$)	0.46	0.66	0.61
S.D.	0.02	0.03	0.06
Aus β-thal spleens ($n = 7$)	0.46	0.68	0.67
S.D.	0.03	0.03	0.08
Normal spleens[a] ($n = 12$)	0.39	0.51	0.56
S.D.	0.09	0.10	0.14
Thai β-thal/Hb E livers ($n = 4$)	0.48	0.66	0.58
Range	0.47–0.49	0.64–0.70	0.53–0.68
Normal livers[a] ($n = 12$)	0.45	0.60	0.64
S.D.	0.06	0.10	0.16
Thai β-thal/Hb E pancreas ($n = 3$)	0.47	0.64	0.58
Range	0.47–0.48	0.60–0.66	0.52–0.62

δ is the center shift in mm/s, ΔE_Q is the quadrupole splitting in mm/s and Γ is the full linewidth at half-height in mm/s.
[a]Taken from [16].

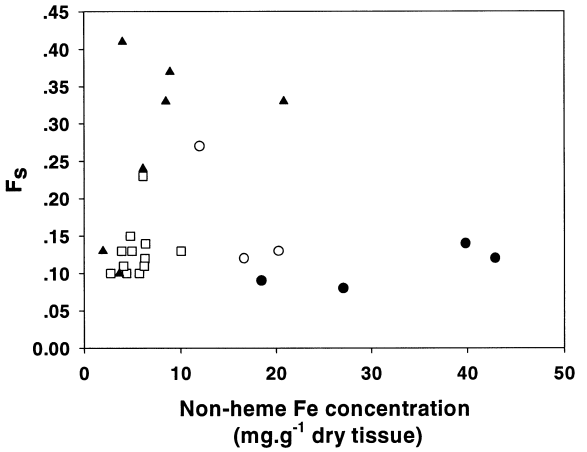

Fig. 2. The fraction, F_s, of the non-heme Mössbauer signal in the form of sextet versus non-heme iron concentration in the tissues. □, Thai β-thalassemia/Hb E spleen; ▲, Australian β-thalassemia spleen; ●, Thai β-thalassemia/Hb E liver; ○, Thai β-thalassemia/Hb E pancreas.

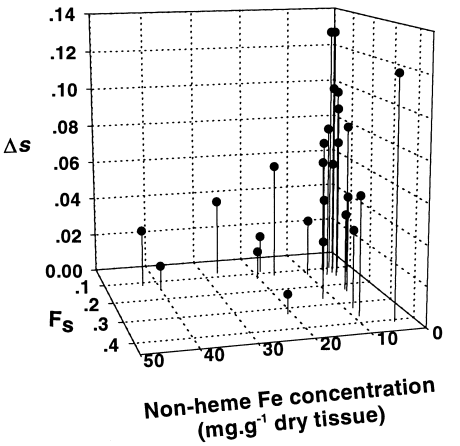

Fig. 3. The calculated error, Δs, on the fraction, s, of the Mössbauer signal in sextet form against non-heme iron concentration in the tissues and the fraction, F_s, of the non-heme Mössbauer signal in the form of sextet in each tissue.

total spectral area with the exception of two samples that gave 14 and 20% respectively. Thus in general, F_s was approximately equal to the fraction, s, of the total Mössbauer signal in the form of a sextet.

A K-S test on the distributions of F_s in the two groups showed a significant difference between the two distributions (K-S statistic = 0.71, $P = 0.01$).

Fig. 2 shows a scatterplot of F_s against the non-heme iron concentration for the tissue samples. Neither the data for the Thai β-thalassemia/Hb E nor the data for the Australian β-thalassemia spleens showed a significant correlation between F_s and the iron concentration. However, when all of the spleen data are taken together, there is a small (Spearman rank-order correlation coefficient of 0.46) but significant ($P = 0.05$) positive correlation between F_s and non-heme iron concentration.

In order to obtain an approximate estimate of the error on each measurement of F_s, each spectrum was analyzed in terms of the total number of counts accumulated in three regions of the spectrum (see Section 2) giving an alternative measure of s and an estimate for the error (Δs) on s. Fig. 3 shows a plot of the calculated error on s for samples with different F_s and non-heme iron concentrations. Although the errors can be reduced to some extent by extending the counting time in the experiment, there is a practical limit to the time for one measurement (approx. 1 week). The exponent of 1/2 in Eq. 1 results in diminishing returns in error reduction for longer counting times. The trends in Fig. 3 are as expected with smaller errors for higher non-heme iron concentrations and larger F_s.

Fig. 4 shows the distribution of differences (each normalized to the calculated error, Δs) in the meas-

Table 3
Mean values and ranges for Mössbauer spectral parameters of sextet components in thalassemic tissues at 78 K

Tissue	δ	ΔE_Q	B_{hf}	Γ
Spleen ($n = 4$)	0.47	−0.21	47.0	0.86
Range	0.42–0.51	−0.28 to −0.13	46.5–47.4	0.73–0.99
Liver ($n = 4$)	0.51	−0.30	46.7	0.81
Range	0.48–0.56	−0.34 to −0.27	46.5–47.0	0.60–1.09
Pancreas ($n = 3$)	0.43	−0.10	46.7	0.86
Range	0.35–0.49	−0.29 to 0.24	46.6–46.8	0.74–1.10

δ is the center shift in mm/s, ΔE_Q is the quadrupole perturbation, B_{hf} is the magnetic hyperfine-field splitting in T, and Γ is the full linewidth at half-height of the outer lines of the sextet in mm/s.

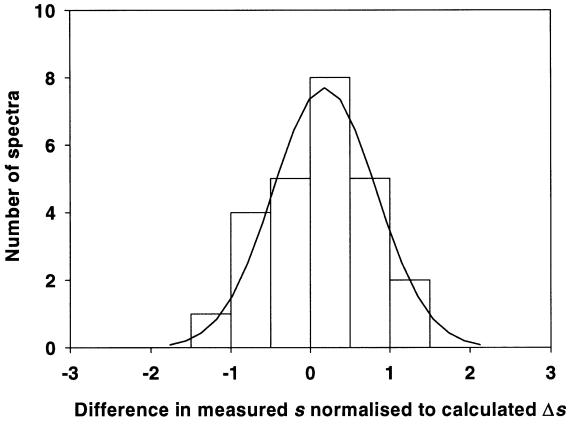

Fig. 4. Histogram showing the distribution of differences (normalized to the calculated Δs) between the value of s measured by the fitting of the standard sextet method and by the region of interest method. The solid curve shows a fit of a Gaussian curve to the distribution.

urement of s between the two methods for all of the tissues in the study. After rejecting one outlier with a difference of 4.7 Δs, the distribution approximates well to a normal distribution. The distribution has a mean of 0.18 ± 0.13 with a standard deviation of 0.65. This indicates that Δs is a reasonable estimate for the random errors on the measurement of s and hence F_s.

Iron concentrations (Table 1) and Mössbauer spectra were measured for four liver and three pancreas samples from Thai β-thalassemia/Hb E patients. Fitting doublets and sextets to the Mössbauer data gave mean spectral parameters as shown in Tables 2 and 3. Previously reported data are also given for normal livers [16]. The spectra were also fitted with the standard spleen sextet. Both methods gave the same value for s within the experimental error, Δs. Values of F_s for the Thai β-thalassemia/Hb E liver and pancreas samples are given in Table 1 and are plotted against the non-heme iron concentration in Fig. 2.

4. Discussion

The thalassemic spleen samples in this study had significantly higher total iron and non-heme iron concentrations than those found for normal spleens. This is consistent with findings of previous workers [17,18]. Our measured values of 5.5 (S.D. 1.7) and 7.8 (S.D. 6.0) mg Fe/g dry tissue for the Thai β-thalassemia/Hb E and Australian β-thalassemia spleens respectively fall between 3.6 (S.D. 1.2) measured for 20 Thai β-thalassemia/Hb E spleens [17] and 10.4 (S.D. 5.8) measured for 7 Italian β-thalassemia major spleens [18]. Interestingly, we did not detect a significant difference in either the total iron concentrations or the non-heme iron concentrations between the Thai β-thalassemia/Hb E spleens and the Australian β-thalassemia spleens despite the fact that the Australian patients are treated with regular blood transfusions and chelation therapy.

The Mössbauer spectra recorded for all of the tissues in this study consisted of a central doublet characteristic of high-spin Fe(III) superposed on a lower intensity sextet, the sextet being due to magnetic hyperfine-field splitting of the ^{57}Fe nuclear energy levels (Tables 2 and 3). Occasionally a second doublet that could be attributed to heme iron was also present. Similar central doublet and sextet signals have been observed in previous reports of Mössbauer spectra of human tissues or extracts from human tissues [5,11–16,19–27]. On the basis of these previous reports these spectral components can be identified as being due to polynuclear iron(III) oxyhydroxide deposits in the tissue. This is consistent with the fact that the predominant form of iron found in iron loaded tissues is usually hemosiderin [7]. The doublet component in the spectrum has parameters consistent with (a) ferritin, (b) non-crystalline hemosiderin, (c) hemosiderin based on the structure of the mineral ferrihydrite, or (d) the doublet component associated with hemosiderin based on the structure of the mineral goethite (the doublet component being due to those hemosiderin particles with a smaller magnetic anisotropy energy) [5,10,11]. As such it is not possible to unambiguously identify the source of this signal and the signal may be due to a combination of the above forms of iron. However, the sextet component in the spectrum can be identified as being due to the presence of hemosiderin based on the structure of the mineral goethite since this is the only form of tissue iron deposit known to give a Mössbauer spectral sextet component with these parameters at 78 K [12–14].

The observed differences in the distributions of Mössbauer spectral parameters of the central doublet between the different tissue groups are suggestive of

differences in the structure of the iron oxide particles within the tissues. For example, the quadrupole splittings of Mössbauer spectral doublets of ferrihydrite are negatively correlated with the degree of crystallinity such that the poorer the crystallinity, the larger the quadrupole splitting [28]. Thus, the generally larger quadrupole splittings of the doublets for the thalassemic spleens compared with those for the normal spleens may possibly be due to the presence of ferrihydrite with a lower degree of crystallinity. However, the difference should be treated with caution since the normal spleen Mössbauer spectra tended to have a lower signal to noise ratio and a larger fraction of heme signal and hence the differences may be, in part, an artefact of the curve fitting procedure [16].

Fig. 3 indicates that for tissue samples with non-heme iron concentrations less than approx. 1 mg Fe/g dry weight tissue, the limit of detection of F_s by Mössbauer spectroscopy is $F_s \approx 0.10$. Since normal spleens typically have iron concentrations of about 1 mg Fe/g dry weight tissue [16,29] and no sextet has been detected in the Mössbauer spectra of normal spleens at 78 K [16] using conventional methods of spectral curve fitting, this implies that F_s must be less than 0.10 in normal tissues, if it is present at all. However, we have confidently detected sextet signals in the thalassemic spleen samples with significantly higher values of F_s being measured in the Australian β-thalassemia spleens than the Thai β-thalassemia/Hb E spleens (Table 1 and Fig. 2). This indicates that the fraction of non-heme iron in the goethite-like form is significantly greater in the Australian β-thalassemia spleens than in the Thai β-thalassemia/Hb E spleens. The small positive correlation of F_s with non-heme iron concentration in the spleen tissues suggests that the fraction of non-heme iron in the goethite-like form may be weakly associated with tissue non-heme iron concentrations.

The lower number of samples of liver and pancreas available for study does not enable a rigorous statistical analysis. However, the Mössbauer spectra were qualitatively similar to those obtained from the spleen samples (Tables 2 and 3) while the non-heme iron concentrations were generally higher than those for the spleen samples (Table 1 and Fig. 2). The low values of F_s measured for the liver samples, despite their higher non-heme iron concentrations, implies that non-heme iron concentration is not the only factor affecting the fraction of non-heme iron in the goethite-like form in human tissues. Cell type could also play a role.

There could be several reasons for the difference in chemical speciation of spleen iron between the two groups of thalassemia patients studied including (a) the different genotypes of the two groups, (b) the effect of blood transfusions, (c) the effect of chelation therapy, (d) differences in diet. We are currently undertaking experiments on animal systems in order to identify which factors may have the most influence on the speciation. The fact that there is different chemical speciation of iron in tissues between different identifiable groups of patients has implications for the treatment and management of iron overload diseases. The reactivities of the different iron oxide forms are expected to be different. Ferrihydrite is less stable than goethite. Its solubility product $(Fe^{3+})\cdot(OH)_3$ is about 10^{-38} to 10^{-39} compared with 10^{-42} to 10^{-44} for goethite [30]. Although the goethite-like form of hemosiderin is likely to be more soluble than goethite itself, hemosiderin from transfusional iron loaded patients has been shown to release iron less readily than the ferrihydrite cores of ferritin when exposed to various chelators [31–33]. Thus the ferrihydrite form of hemosiderin may be more toxic to cells than the goethite-like form on an atom for atom basis because of its higher solubility. On the other hand, it should be easier to chelate and remove from the body than the goethite-like form. This may partly explain why the Australian patients in our study had higher fractions of their non-heme spleen iron in the goethite-like form. More of the ferrihydrite form may have been removed by the chelation therapy, the remaining deposits thus being enriched in the goethite-like form.

Acknowledgements

This work was supported by the National Health and Medical Research Council (Australia). Wanida Chua-anusorn was supported by a Murdoch University Postgraduate Research Award. We would like to thank the patients and clinicians from Siriraj Hospital, Bangkok and Princess Margaret Hospital,

Perth for making available the tissue samples for study.

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